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BRIEF REPORT

CINP

β_2 Nicotinic acetylcholine receptor availability in post-traumatic stress disorder

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Abstract

Availability of nicotinic acetylcholine receptors containing β_2 subunits (β_2-nAChRs) was studied in unmedicated, symptomatic patients with post-traumatic stress disorder (PTSD) and healthy control subjects, all current non-smokers. A subgroup of participants had a history of smoking. Availability of β_2-nAChRs in the mesiotemporal cortex, prefrontal cortex, thalamus and striatum was determined using the radiotracer [^{123}I]5-IA-85380 ([^{123}I]5-IA) and single-photon emission computed tomography (SPECT). PTSD symptoms were assessed using the Clinician-Administered PTSD Scale (CAPS). Never-smoking PTSD patients compared to never-smoking healthy controls showed significantly higher [^{123}I]5-IA binding in the mesiotemporal cortex (ANOVA: $F=6.21$, d.f.$=1, 11$, $p=0.030$). Among all PTSD patients, there was a significant correlation between the re-experiencing symptom cluster and thalamic [^{123}I]5-IA binding ($R^2=0.66$, $p=0.019$, Bonferroni corrected). These findings not only suggest an involvement of β_2-nAChRs in the pathophysiology of PTSD but also raise the possibility that this receptor may be a novel molecular target for drug development.

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Introduction

Memory deficits and hyperarousal are defining symptoms of post-traumatic stress disorder (PTSD) (APA, 1994). Typically, PTSD patients can have difficulties in actively recalling aspects of the traumatic event that caused the disorder, while on the other hand they can intrusively re-experience distressing memories of the trauma, they have difficulties in concentrating, and they can also show persistent symptoms of increased arousal, not present before the trauma. The neurobiological basis of these symptoms remains unclear.

Although nicotinic acetylcholine receptors (nAChRs) have been strongly implicated in memory dysfunctions and regulation of arousal (Gotti et al., 1997; Levin et al., 2006), they have never been studied in PTSD. This is unexpected since there is a growing body of indirect evidence implicating nAChRs in the neurobiology of PTSD. A link between nAChRs and PTSD may also at least partially explain the increased risk for nicotine dependence among individuals diagnosed with PTSD (Breslau et al., 2003). On a neurobiological basis, nAChRs show high expression in brain regions previously implicated in PTSD, including the hippocampus, prefrontal cortex, striatum and thalamus (Frewen and Lanius, 2006; Gotti et al., 1997; Lanius et al., 2001, 2004).

Animal studies have shown that exposure to immobilization stress alters the levels of brain nAChR expression (Takita and Muramatsu, 1995). However, immobilization stress may not constitute a relevant model for PTSD in humans. PTSD patients typically show an enhanced inhibition of the hypothalamic–pituitary–adrenal (HPA) axis, which has been reliably reproduced by a single-prolonged stress (SPS) model in rats (Kohda et al., 2007). nAChR expression has not

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yet been studied in this model, but self-administration of nicotine in rats has been shown to modulate the sensitization of the HPA axis to stressors (Chen et al., 2007).

The selective nAChR agonist radioligand [^{123}I]5-IA-85380 ([^{123}I]5-IA), permits in-vivo assessment of central binding of nAChRs containing the β_2 subunit (β_2-nAChRs), using single photon emission computed tomography (SPECT). In the present study, we determined the density of brain β_2-nAChRs, using [^{123}I]5-IA and SPECT in a group of unmedicated, symptomatic PTSD patients and individually matched healthy control subjects (HC), all non-smokers. Regions of interest (ROIs) were the mesiotemporal cortex, prefrontal cortex, striatum and thalamus, chosen as primary ROIs based upon their relevance to the pathophysiology of PTSD and high expression of β_2-nAChRs (Frewen and Lanius, 2006; Gotti et al., 1997; Lanius et al., 2001, 2004). We were particularly interested in the relationship between [^{123}I]5-IA binding and PTSD re-experiencing and hyperarousal symptoms given the hypothesized relevance of nAChRs in the aetiology of these characteristic symptoms of PTSD.

Method

Subjects

Ten PTSD patients (seven female, mean ± S.D. age 41.8 ± 13.0 yr) and ten age- and gender-matched HC (seven female, mean ± S.D. age 39.4 ± 14.5 yr) were included in the study. Patients had developed PTSD in consequence of sexual abuse ($n=3$), sexual assault ($n=1$), physical abuse ($n=2$), combat-related experience ($n=2$), car accident ($n=1$) and man-made disaster ($n=1$).

Subjects were recruited by public advertisement. Written informed consent was obtained from all subjects after study procedures had been fully explained. The study was approved by the Yale University School of Medicine Human Investigation Committee, the West Haven Veterans Administration Human Subjects Subcommittee and the Yale Radiation Safety Committee.

PTSD diagnosis and diagnosis of comorbid psychiatric disorders were established using the Structured Clinical Interview for DSM-IV Axis I Disorders. PTSD symptoms were evaluated using the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995). CAPS scores measuring re-experiencing symptoms (CAPS-B) and hyperarousal symptoms (CAPS-D) were used for further analysis, according to the hypotheses of the study. Comorbid disorders in PTSD patients included current major depression ($n=4$), past but not current major depression ($n=2$), past but not current alcohol abuse ($n=3$), and past but not current eating disorder ($n=1$). HC were free of any current or past psychiatric disorder based on DSM-IV criteria. Depressive symptoms in PTSD patients were evaluated using the 24-item Hamilton Depression Rating Scale (HAMD).

All subjects were evaluated by physical examination, electrocardiogram, standard laboratory tests, urinanalysis and toxicology. Subjects with any significant medical or neurological condition, as well as with a history of head injury were excluded from the study. No use of medication was allowed during the study.

Smoking history was assessed by self-reports and the Fagerstrom Test for Nicotine Dependence. None of the study subjects indicated having been smoking in the 12 months prior to entering the study. Seven HC and six PTSD patients indicated that they were never-smokers, whereas three HC and four PTSD patients had a variable history of smoking. Repeated urine cotinine tests were performed at initial screening as well as on the SPECT scan days to independently verify non-smoking status for ~3 wk preceding the SPECT scan.

Magnetic resonance (MR) images were obtained before the SPECT scan to provide an anatomical framework for analysis (1.5 T Sigma camera, echo time = 5 ms, repetition time = 25 ms, number of excitations = 2, matrix = 256 × 256 pixels, field of view = 16 cm). Abnormal MR image findings were regarded as exclusion criteria for the study. MR images were similar across the groups. On the SPECT scan day, menstrual cycle phase was recorded by self-report of the first day of the last menses and PTSD subjects were matched with HC.

SPECT imaging

[^{123}I]5-IA SPECT imaging was performed as described in detail elsewhere (Staley et al., 2005). In brief [^{123}]5-IA was administered in a bolus plus constant infusion paradigm with a bolus:infusion ratio of 7.0 h. HC were administered an average ± S.D. bolus amount of 156 ± 14 MBq and an average ± S.D. infusion dose of 201 ± 17 MBq. PTSD patients received an average ± S.D. bolus of 157 ± 13 MBq and an average ± S.D. infusion dose of 203 ± 16 MBq. Three SPECT scans, 30 min each, were obtained between 6 h and 8 h after the beginning of the infusion. Plasma samples were collected immediately before the first and after the last scan for

quantification of total and free fraction of parent tracer in the plasma.

Image analysis

SPECT emission images were filtered using a 3D Butterworth filter (order 10; cut-off frequency 0.24 cycles/pixel) and reconstructed using a filtered back-projection algorithm with a ramp filter and a 128×128 matrix to obtain 50 slices with a pixel size of $2.06 \times 2.06 \times 3.56$ mm in the x-, y-, and z-axes. Non-uniform attenuation correction was performed. Each subject's MRI was co-registered to the SPECT image using SPM2 (Wellcome Department of Cognitive Neurology, University College London, London, UK). 3D volumes of interest were generated based upon the MR images for each predefined ROI and transferred to the co-registered SPECT image to determine regional radioactive densities (counts per minute/pixel). Regional β_2-nAChR availability was determined by V_T' (regional activity/total plasma parent), a highly reproducible outcome measure (Staley et al., 2005). The mesiotemporal cortex, prefrontal cortex, striatum and thalamus were entered into the analysis as primary ROIs.

Data analysis

Differences between HC and PTSD subjects in regional [^{123}I]5-IA binding were analysed using multivariate ANOVA, with V_T' values for the primary ROIs entered as dependent variables. Separate analyses were performed for subgroups of never-smokers. Correlations between regional V_T' values and CAPS scores in PTSD patients were analysed using Pearson's coefficients calculated on normally distributed data. Testing for normal distribution was performed using the Kolmogorov–Smirnov test. Correlation analyses between CAPS scores and regional V_T' values were corrected for multiple testing with a Bonferroni factor of 4, according to the four a-priori selected primary ROIs. All tests were performed two-tailed, results were considered significant at $p < 0.05$ and corrected p values are reported.

Results

Multivariate ANOVAs considering all primary ROIs showed a significant difference in [^{123}I]5-IA binding between never-smoking HC and PTSD patients ($F = 4.29$, d.f. = 4, 8, $p = 0.038$). Between-group differences were most pronounced in the mesiotemporal cortex where never-smoking PTSD patients relative to never-smoking HC showed significantly higher [^{123}I]5-IA binding (ANOVA: $F = 6.20$, d.f. = 1, 11, $p = 0.030$, Figure 1). We also found a non-significant trend towards higher [^{123}I]5-IA binding when comparing the total group (including four PTSD patients and three HC with smoking history) of PTSD patients vs. HC ($F = 2.91$, d.f. = 4, 14, $p = 0.057$).

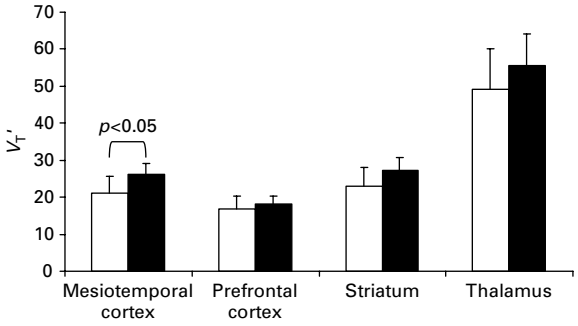

Figure 1. Never-smoking PTSD patients (■) compared to never-smoking healthy controls (□) showed significant higher [^{123}I]5-IA binding in the mesiotemporal cortex (ANOVA: $F = 6.21$, d.f. = 1, 11, $p = 0.030$; MANOVA: $F = 4.29$, d.f. = 4, 8, $p = 0.038$). Shown are mean and standard deviation of regional [^{123}I]5-IA binding, as determined by V_T' (regional activity/total plasma parent).

[^{123}I]5-IA binding was independent of age, gender, and laterality. Mean $\pm$ S.D. CAPS-B and CAPS-D scores in PTSD patients were 20.2 ± 7.1 and 20.4 ± 5.6, respectively. Within the total group of PTSD patients, a significant correlation was found between the CAPS-B score and [^{123}I]5-IA uptake in the thalamus ($R^2 = 0.66$, $p = 0.019$, Figure 2).

In PTSD patients [^{123}I]5-IA binding did not correlate with CAPS-D scores or HAMD scores in any ROI. [^{123}I]5-IA binding in PTSD patients was further independent of a diagnosis of current or lifetime major depression (MANOVA: $F = 0.78$, d.f. = 4, 5, $p = 0.58$ and $F = 0.98$, d.f. = 4, 5, $p = 0.51$, respectively) or diagnosis of past alcohol abuse (MANOVA: $F = 1.74$, d.f. = 4, 5, $p = 0.27$).

Discussion

Never-smoking PTSD patients relative to never-smoking HC showed significantly higher β_2-nAChR availability in the mesiotemporal cortex. This significant difference was weakened when people with previous exposure to nicotine were included in the analysis. There was only a non-significant trend for elevated β_2-nAChR availability in the total group of PTSD patients vs. HC. Among the PTSD patients, thalamic β_2-nAChR availability showed 66% shared variance with re-experiencing symptoms, as

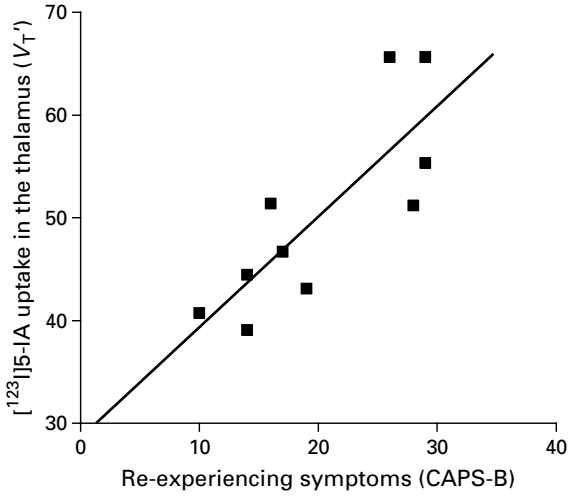

Figure 2. A significant correlation ($R^2 = 0.66$, $p = 0.019$, Bonferroni corrected) was found between thalamic [^{123}]5-IA uptake and re-experiencing symptoms (CAPS-B score) in PTSD patients ($n = 10$).

expressed in a significant correlation between CAPS-B scores and [^{123}I]5-IA binding.

The data for the first time supports an involvement of β_2-nAChRs in PTSD. Our finding of a significant difference between PTSD patients and HC in [^{123}I]5-IA binding was most prominent in never-smoking individuals. In a previous post-mortem study differences between non-smokers and former smokers who had quit at least 2 months before death were indicated for hippocampal and thalamic [^{3}H]nicotine binding (Breese et al., 1997). It is therefore possible, that exclusion of nicotine administration in the 3 wk preceding the SPECT scan may not have been sufficient to exclude an interfering effect of non-indicated nicotine administration shortly before, which may occur rather in individuals with variable smoking history than in never-smokers.

The mesiotemporal cortex includes two regions which have been consistently implicated in the neurocircuitry of PTSD: the amygdala and hippocampus (Shin et al., 2006). Both regions have been shown to play a critical role in memory and may be involved in memory deficits shown by PTSD patients (Ehlers et al., 2004). Decreases in hippocampal volume in PTSD patients have been shown to be inversely associated with verbal memory deficits (Shin et al., 2006), and infusion of $\alpha_4\beta_2$ nAChR antagonists in the amygdala and hippocampus both produced working-memory impairments in rats (Levin et al., 2006). The mesiotemporal cortex is anatomically and functionally closely connected to the thalamus which also has been implicated in PTSD (Lanius et al., 2001). We found not only the highest concentrations of β_2-nAChR in the thalamus but also, more importantly, a significant association between thalamic [^{123}I]5-IA uptake and the re-experiencing syndrome cluster of PTSD. This adds to our emerging understanding of the distinct neurobiology of PTSD symptom clusters. We previously hypothesized that the thalamus might contribute to PTSD symptoms as a consequence of its gating of access of sensory information to the cortex, both in the context of PTSD-associated vivid trauma-related memories and trauma-related nightmares (Krystal et al., 1995). In support of this hypothesis, patients with PTSD show reduced activation of this region while being exposed to narratives of their psychological traumatization (Lanius et al., 2001). nAChRs modulate the vividness of dreams (Page et al., 2006) and play an important role in learning and memory (Gotti and Clementi, 2004). The current data raise the possibility that β_2-nAChRs contribute to re-experiencing symptoms in PTSD by modulating the sensory input to the cortex and by modulating cortical neuroplasticity associated with learning and stress response.

Altogether, this first in-vivo imaging study of nAChRs in patients with PTSD implicates β_2-nAChRs in the pathophysiology of the disorder. Previous neuroreceptor studies in PTSD have reported reduced cortical benzodiazepine receptor binding sites in two of three studies (Bremner et al., 2000; Fujita et al., 2004; Geuze et al., 2007) and no changes in 5-HT_{1A} receptor binding in these patients (Bonne et al., 2005).

The relatively small sample size of the current study calls for a replication of these preliminary findings in a larger and more heterogeneous sample with regard to smoking history, sex and age. The present study also lacked the statistical power to replicate the recent finding of a decline of β_2-nAChR availability with age (Mitsis et al., 2007). In order to determine the potential role of nAChRs in the development of PTSD, future studies should address the impact of extreme stress exposure on nAChR expression in people who were resilient to the psychological consequences of trauma. Finally, future studies are needed to clarify the potential role of β_2-nAChRs in the comorbidity of smoking and PTSD, an important clinical problem (Rasmusson et al., 2006). The findings of the present study also raise the possibility that this receptor may be an interesting candidate for drug development. This could be an important step towards improvement of treatment options for patients with PTSD given the evidence that currently available treatments are

effective only in subgroups of patients with PTSD (Stein et al., 2006).

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Statement of Interest

Dr Seibyl has served as consultant for GEHC, Boston Life Sciences, Eisai, and has equity ownership of Molecular Neuroimaging, US. Dr Krystal has served as a paid scientific consultant to Astra-Zeneca, Bristol–Myers Squibb, Cypress Bioscience, Eli Lilly, Forest Laboratories, GlaxoSmith-Kline, Janssen Research Foundation, Merz Pharmaceuticals, Organon Pharmaceuticals, Pfizer Pharmaceuticals, Shire Pharmaceuticals, Takeda Industries, UCB Pharma and US Micron. Dr Neumeister has received grant support from Eli Lilly, UCB Pharma and Pfizer Pharmaceuticals.

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